Chemosphere, Vol.24, No.12, pp 1911-1919, 1992
Printed in Great Britain

0045-6535/92 $5.00 + 0.00
Pergamon Press Ltd.

BIOCONCENTRATION OF *trans*-CHLORDANE BY THE MIDGE, *Chironomus decorus*

G. A. Harkey*[1] and S. J. Klaine
*Department of Biology, Memphis State University,
Memphis, TN 38152
and
Institute of Wildlife and Environmental Toxicology, Clemson University,
Pendleton, SC 29670

ABSTRACT

The accumulation and transport of the organochlorine pesticide, *trans*-chlordane, by the midge, *Chironomus decorus* was examined in a whole life cycle laboratory exposure assay. Larvae were kept in dosed water exposures including food particles from egg stages through adult metamorphosis and were sampled for weight gain and chemical content over the course of the 50 day study. Accumulation of *trans*-chlordane was related to the amount of contaminant in the larval environment. Concentration of *trans*-chlordane (ng contaminant/g tissue) in larvae was not significantly different from second through fourth instar stages but depended upon the weight of the larvae. Differences in time to metamorphosis, weight gain and sex ratios occurred among all replicate exposures, including controls, suggesting nongenetic maternal differences, rather than environmental variation caused by chemical exposure. When the larvae metamorphosed, an average of 82.6% of the contaminant was retained in the adults while 11.4% was left behind in the shed exuviae. This study suggests that terrestrial predators feeding on emerging aquatic insects can be exposed to organic pesticides from aquatic environments.

INTRODUCTION

Midges are an important part of the food chain, providing a source of nutrients for both aquatic and terrestrial predators. *Chironomus* sp. larvae represent a large portion of the total benthic fauna in many freshwater lakes and streams, with estimates of 2,500 larvae/m^2 in some areas of Lake Michigan (Nalepa *et al.* 1985), and 10,000 - 40,000 larvae/m^2 in Lake Esrom, Denmark (Jonasson 1972). Larval accumulation of contaminants from sediments and water may act as a link between pollutants in the aquatic and terrestrial environments when these large populations undergo metamorphosis. In order to investigate this process, we measured accumulation of a persistant organochlorine insecticide, *trans*-chlordane, in the midge, *Chironomus decorus*, over an entire life cycle. Research objectives were to 1) determine accumulation of *trans*-chlordane in *C. decorus* from early larval stages to adult metamorphosis in a static renewal system, 2) determine differences in time to metamorphosis, sex ratios, and weight gain between *trans*-chlordane exposed groups and control groups, and 3) determine relative amounts of *trans*-chlordane accumulated in larvae, exuviae, and adults to estimate the amount transported out of the aquatic environment.

MATERIALS AND METHODS

Radiolabeled [^{14}C] *trans*-chlordane (specific activity of 13.7 mCi/mmol) was dissolved in methanol and was found to be >98.0% pure via thin layer chromatography. Nine replicate exposure containers were prepared containing three different aqueous concentrations of ^{14}C-*trans*-chlordane: low (0.69 $\pm$ 0.09 ng/L), medium (1.03 $\pm$ 0.23 ng/L), and high (1.37 $\pm$ 0.06 ng/L). Three control containers were also prepared.

---

[1] Current address: Great Lakes Environmental Research Laboratory (NOAA), 2205 Commonwealth Blvd., Ann Arbor, MI 48104

Exposure containers were 250 ml teflon centrifuge bottles filled to capacity with either dosed water or water with methanol carrier that served as controls. All water used in the study was reconstituted moderately-hard water. On day 1, one egg mass of *C. decorus* was added to each bottle. All added egg masses were approximately the same size and contained 200-300 eggs. All bottles were equipped with screw-top caps and individual air lines and were kept in an environmental chamber at 20 C on an 18:6 light:dark cycle. On day 3, 20 mg of Cerophyl was added to each bottle as a food source and tube-building material. Twenty mg Cerophyl was added to each bottle three times per week thereafter for the duration of the study. Once per week, 80 ml of water was removed from each bottle and was replaced with 80 ml of reconstituted moderately-hard water dosed with the corresponding concentration of radiolabeled *trans*-chlordane. This water was prepared on the same day as its use and was checked for contaminant concentration prior to addition to the exposure bottles. Control bottles were handled in the same manner as test bottles, which included a weekly removal of 80 ml water with a subsequent addition of 80 ml reconstituted moderately-hard water containing carrier only. Unfiltered water samples from all test bottles were analyzed weekly for toxicant concentration via liquid scintillation counting (LSC). Each week, two to eight larvae were taken at random from each bottle, dipped into clean water, blotted dry, weighed, and analyzed for *trans*-chlordane content. All analyses for ^{14}C-*trans*-chlordane in larvae, exuviae, and adults were determined by direct extraction of the contaminant by the scintillation cocktail and LSC, as per Landrum *et al.* (1985). As adults emerged, they were taken from the bottles, weighed, sexed, and analyzed for ^{14}C-*trans*-chlordane concentration via LSC. Exuviae were also removed from exposure bottles, dipped into clean water, blotted dry, weighed, and analyzed for contaminant concentration. Remaining larvae which had not metamorphosed after 50 days were weighed and analyzed for ^{14}C-*trans*-chlordane content in the same manner at the conclusion of the study.

TABLE 1.
CONCENTRATION OF ^{14}C-*trans*-CHLORDANE IN UNFILTERED WATER EXPOSURES (ng/ml)

Day	1	10	17	24	31	38	50
Low Exposure	81.96 ± 12.3	88.91 ± 4.1	148.80 ± 24.6	144.72 ± 28.7	161.53 ± 23.8	166.40 ±16.4	132.63 ± 4.1
Medium Exposure	126.60 ± 16.4	109.00 ± 12.3	177.43 ±36.9	174.65 ± 36.9	199.20 ± 16.4	221.73 ± 12.3	250.34 ± 28.7
High Exposure	113.15 ± 4.1	111.00 ± 16.4	260.23 ± 151.6	227.43 ± 61.5	273.70 ± 49.2	278.23 ± 4.1	293.42 ± 16.4

*Numbers denote mean *trans*-chlordane concentrations in water with added food particles ± 1 S D.

Bioconcentration factors were calculated as the ratio of *trans*-chlordane in tissue (ng/g wet weight) to that in the water (ng/ml) on Day 50. Accumulation of *trans*-chlordane among the low, medium, and high

exposure groups and calculated bioconcentration factors were examined by analysis of variance (ANOVA). If significant differences at $p < 0.05$ were observed in the ANOVA, then a Scheffe's multiple range test was used to discover where the significant differences lay. Student t-tests were used to compare differences in numbers and mass of males and females that emerged from the treatments.

RESULTS

In this study, 295 larvae, 34 exuviae, and 54 adult *C. decorus* were analyzed for ^{14}C-*trans*-chlordane content. Concentrations of *trans*-chlordane in the exposure bottles rose significantly over time (Table 1). This is most likely due to the adsorption of weekly *trans*-chlordane loads to accumulated Cerophyl particles in the water column.

The amount of *trans*-chlordane per individual larvae rose significantly in the high exposures up to day 34 (Table 2). This correlates with an average larval weight increase up to day 40. After day 40, a steady decrease in larval mass (Fig. 1) as well as amount of *trans*-chlordane per individual occurred in all exposures until the end of the study (Table 2). A large variation of *trans*-chlordane content was found in individual midges exposed to low, medium, and high replicate exposures from day 20 to the end of the study. Accumulation of *trans*-chlordane tended to rise with mass in individual larvae ($r^2 = .74$, Fig. 2). However, larval concentration of *trans*-chlordane (ng contaminant/g wet tissue) remained relatively constant from second through fourth instar stages in low, medium, and high *trans*-chlordane exposures (Table 3). There were no significant differences in larval mass among dose levels over the course of the study. Larvae exposed to high *trans*-chlordane concentrations were the only group that showed a significant increase in the

TABLE 2.
AMOUNT OF *trans*-CHLORDANE IN LARVAE FROM LOW, MEDIUM, AND HIGH EXPOSURE BOTTLES, DAY 20 TO DAY 50 (ng/individual)*

Day	Low	Medium	High
20	17.2 ± 4.1	15.0 ± 0.9	56.5 ± 55.7
24	31.5 ± 33.0	14.8 ± 5.7	154.4 ± 243.2
31	72.9 ± 61.7	52.6 ± 16.0	204.1 ± 300.3
34	-**	-	481.2
40	105.7	-	-
41	81.7	-	-
44	54.7 ± 14.6	-	-
45	35.9	99.2 ± 29.5	168.4 ± 100.3
50	42.9 ± 6.0	101.7 ± 27.4	58.7 ± 3.7

* Numbers denote ± 1 SD, n = 3 - 8 individual larvae
** No data available

amount of contaminant per individual accumulated over time (ANOVA: $F = 6.598$; $df = 1,13$; $p = 0.0234$).

A direct relationship was found between *trans*-chlordane concentration in the water and larvae,

exuviae, and adults when calculated on a concentration basis, (micrograms/g wet tissue; Figure 3). Significant differences in bioconcentration factors (BCF) occurred among larvae, exuviae, and adults (ANOVA: $F = 12.204$; $df = 2, 123$; $p = 0.0001$), and among the low, medium, and high exposure groups (ANOVA: $F = 7.917$; $df = 2, 123$; $p = 0.0006$; Table 4). BCFs increased significantly in adults, compared with larvae and

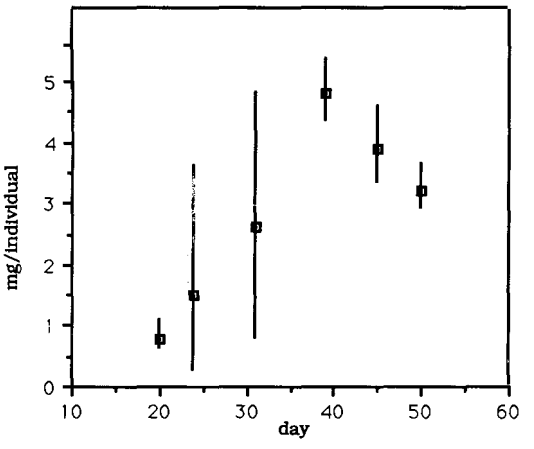
TABLE 3.
CONCENTRATION OF *trans*-CHLORDANE IN LARVAE FROM LOW, MEDIUM, AND HIGH EXPOSURE BOTTLES, DAY 20 TO DAY 50
(micrograms *trans*-chlordane/gram wet tissue)*

Day	Low	Medium	High
20	24.6 ± 5.1	31.4 ± 4.2	54.4 ± 18.1
24	19.4 ± 4.4	22.9 ± 5.2	39.1 ± 12.0
31	21.7 ± 5.4	21.9 ± 1.0	52.9 ± 16.6
34	-**	-	71.0 ± 18.6
40	18.9 ± 1.3	-	-
41	18.0	-	-
44	13.2 ± 9.0	-	-
45	12.4	24.3 ± 7.4	33.7 ± 6.7
50	12.5 ± 5.9	30.2 ± 7.3	24.7 ± 2.3

* Numbers denote ± 1 SD, n = 3 - 8 individual larvae
** No data available

FIGURE 1.

LARVAL MASS, DAY 20 TO DAY 50

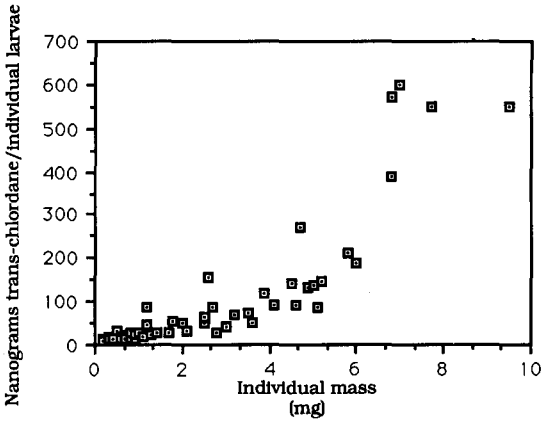


error bars denote ± 1 SD
n = 295

exuviae. BCFs were also higher for the high concentration exposure relative to low and medium exposures (Table 4).

There were considerable differences among exposures for weight gain of the larvae and time to metamorphosis. For example, on day 17, mean larval weights in the three exposure replicates were 0.325 ± 0.28 mg (± 1 SD, wet weight) for the low exposures, 0.288 ± 0.57 mg for the medium exposures, 1.119 ± 1.53 mg for the high exposures, and 0.221 ± 0.10 mg for the control group. Time to metamorphosis also varied among exposures of the same concentration, as well as among controls. Adult emergence ranged from day 35 to day 50+ in the low exposures, day 36 to day 50+ in the medium exposures, day 29 to day 50+ in the high exposures, and day 29 to day 50+ in controls (the study was concluded on day 50). A total of 17 to 19 adults were collected from each of the three treatments. The total numbers of larvae taken from the three treatments ranged from 63 in the high exposure to 107 in the medium exposure.

FIGURE 2.
RELATIONSHIP OF LARVAL MASS TO AMOUNT OF *trans*-CHLORDANE ACCUMULATED IN INDIVIDUALS FROM LOW, MEDIUM, AND HIGH EXPOSURE GROUPS



n = 69

Sex ratios in emerging adults were approximately 1:1 in the three exposure groups and controls, where a total of 21 females and 27 males were analyzed. A significant difference in mass was found between the two sexes (females = 2.51 ± 1.0 mg; males = 1.43 ± 0.4 mg; $t = 4.985$; $df = 46$; $p < 0.05$). Mean *trans*-chlordane concentrations in tissues were not significantly different between male and female adults (females = 149.45 nmol/g; males = 171.87 nmol/g; $t = 0.788$; $df = 46$; $p > 0.05$).

Most of the *trans*-chlordane accumulated in the larvae was retained in adults after metamorphosis (Table 5). From mass balance calculations for larvae, exuviae, and adults, an average of 82.6% of the *trans*-chlordane accumulated in larval tissue was retained in adults, while 11.4% was left behind in the exuviae. The percentages of contaminant retained in adults and left behind in exuviae stayed relatively constant among low, medium, and high contaminant exposures.

TABLE 4.

AVERAGE *trans*-CHLORDANE BIOCONCENTRATION FACTORS OBTAINED IN *Chironomus decorus*

	Larvae*	Exuviae	Adults
Low Exposure Group	126.8[a] (29.6)** n = 15	108.7[a] (24.1) n = 10	202.3[b] (126.7) n = 17
Medium Exposure Group	127.4[a] (32.9) n = 16	167.9[a] (67.4) n = 15	214.9[b] (54.2) n = 15
High Exposure Group	175.2[a] (66.0) n = 16	251.0[b] (155.5) n = 9	315.8[c] (180.6) n = 13

* Scheffe's multiple range test. Different superscripts denote significant differences among the larvae, exuviae, and adult groups at $p < 0.05$.
** Numbers in parenthesis are ± 1 S.D.

TABLE 5.

AVERAGE AMOUNT OF *trans*-CHLORDANE MEASURED IN *Chironomus decorus*

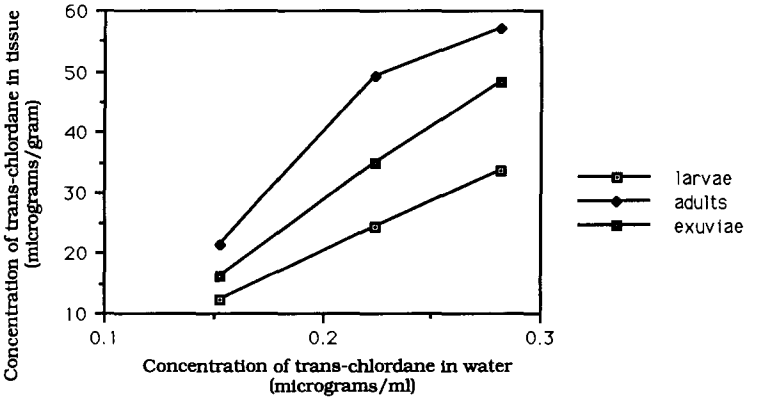
	Larvae	Adult	Exuviae	Amount Retained in Adult*	Amount Left in Exuviae
	(nanograms/individual)				
Low Exposure Group	64.2	59.5	7.0	92.7%	10.9%
Medium Exposure Group	100.5	81.2	10.2	80.8%	10.2%
High Exposure Group	113.6	84.5	14.8	74.4%	13.0%

*Percentages retained and left in *C. decorus* were computed from the mean total amount of compound in individual larvae.

DISCUSSION

The concentration of *trans*-chlordane in larvae, exuviae, and adult *C. decorus* was proportional to the amount of contaminant in the aqueous environment. The static systems used in this study did not contain sediment-bound contaminants, and the increasing concentrations of *trans*-chlordane in the exposure replicates can best be likened to a constant influx of particle-laden contaminants. Such contamination is likely to occur as outflushing from contaminated sediments to adjacent waterways. Therefore, the amount of contaminant biologically available for uptake by *Chironomus* larvae could differ for a natural body of water containing in-place pollutants.

FIGURE 3.
COMPARISON OF *trans*-CHLORDANE CONCENTRATIONS IN UNFILTERED WATER EXPOSURES WITH CONCENTRATIONS IN LARVAE, EXUVIAE, AND ADULTS AT END OF STUDY



Other studies conducted with *Chironomus* species have shown that accumulation of contaminants increases with increasing concentrations in sediments and water. Increased body burdens of zinc, cadmium, and copper in *C. riparius* larvae were found with increasing levels of aqueous exposures (Timmermans and Walker 1989). Upon exposure to PCBs, the uptake by *C. plumosus* larvae, adults, and exuviae was directly related to the PCB concentrations in the sediment (Larsson 1984). Further, insects emerging from the St. Clair and Detroit Rivers contained levels of PCBs and other organics that were highly correlated with sediment concentrations (Ciborowski and Corkum 1988).

In this study, a large variation in the amount of *trans*-chlordane per individual was seen in larval tissue, especially in the high exposure treatments. This variation may have been due to the fact that the larvae taken at random from the bottles at each time point varied in size. This size variation subsequently resulted in a wide variation of total surface areas to which the contaminant could adsorb. While larval concentration of *trans*-chlordane remained relatively constant from day 20 to the end of the study in each level of contaminant exposure, larger animals contained a larger amount of contaminant. The fact that *Chironomus* species accumulate hydrophobic compounds via adsorption to the integument was demonstrated in a study where *C. tentans* were exposed to DDE (Derr and Zabik 1974). When concentrations of DDE in larvae were plotted against their calculated surface areas, a direct relationship was found between the surface area of the larvae and the amount of DDE that had accumulated.

There was little change in the amount of *trans*-chlordane contained in larvae (ng contaminant/g tissue) from second through fourth instar stages within the low, medium, and high exposure groups. By day 20, a maximum concentration in larvae was seen in the low and medium exposure groups, although mean concentration of contaminant in the high exposure group did not reach a maximum until day 34. Larval mass varied most on days 24 and 31. During this time, some of the larvae had already reached the fourth instar stage, while others were still in the smaller third instar stage. As a result, relatively large differences in time to

metamorphosis occurred among different replicates, including the controls. The differences in weight gain and time to metamorphosis among bottles were most likely due to nongenetic maternal quality differences among the separate egg clutches added to the bottles at the beginning of the study (Huffaker and Rabb 1984), and were not due to contaminant exposure.

When the midge larvae metamorphosed to adults, the *trans*-chlordane was concentrated. Bioconcentration factors were highest in adults which had been exposed to the high contaminant concentration. This was also found to be true when *C. plumosus* was exposed to increasing concentrations of PCBs in sediment (Larsson 1984). As in this study, BCFs were greatest in adults exposed to high PCBs, while larvae and exuviae followed the same pattern (Larsson 1984). However, when *C. riparius* was exposed to anthracene in water, BCFs were not affected by increasing concentrations of contaminant (Gerould *et al.* 1983). These data indicate that the ability of a contaminant to bioaccumulate in *Chironomus* larvae may be dependent upon its physical and chemical characteristics and mode of exposure.

The amount of *trans*-chlordane retained in adults was high (74.4 - 92.7%) relative to the amount left behind in exuviae. From this data, it is apparent that the potential for transport of *trans*-chlordane from sediments to the terrestrial environment is high. In a modeling exercise, it was shown that the potential for contaminant removal from an aquatic system was highly significant for areas of moderate to high insect productivity and contaminant bioconcentration factors greater than 1000 (Menzie 1980). For BCFs between 100 and 1000 and the same environmental conditions, significant contaminant removal was also estimated. For the contaminant 2,3,7,8-tetrachlorodibenzofuran, a highly lipophilic compound, a 0.2 to 1.3% annual export by emerging chironomids was calculated for a lake sediment of low primary productivity (Fairchild *et al.* 1992). These estimates would increase in systems possessing a greater emergence biomass. It is possible that *trans*-chlordane could follow a similar pattern in transport from contaminated sediments to the terrestrial environment.

Chlordane follows pathways to the aquatic environment that are characteristic of other organic pesticides. Its low solubility in water and affinity for sediment organic carbon are characteristics that contribute to its removal from original application sites to sediments of lakes and streams. Once in the sediment, *trans*-chlordane is available for accumulation in benthic organisms such as midge larvae. The information in this study suggests that although sediments of lakes and watercourses are thought to be the ultimate sink for hydrophobic pesticides such as *trans*-chlordane, remobilization of the compounds back into the terrestrial food chain is an ongoing process.

ACKNOWLEDGEMENTS

This work was supported by the Department of Biology, Memphis State University, Memphis, Tennessee, and the Great Lakes Environmental Research Laboratory (NOAA), Ann Arbor, Michigan. The Velsicol Chemical Company provided the radiolabelled compound. Critical reviews of the manuscript were provided by P. Landrum and M. Lydy. GLERL contribution #800. Technical contribution #3274, South Carolina Agriculture Experiment Station, TIWET contribution #92002, Clemson University, Clemson, SC.

LITERATURE CITED

Ciborowski, J. J. H. and L. D. Corkum. 1988. Organic contaminants in adult aquatic insects of the St. Clair and Detroit Rivers, Ontario, Canada. J. Great Lakes Res.14(2):148-156.

Derr, S. K. and M. J. Zabik. 1974. Bioactive compounds in the aquatic environment: Studies on the mode of uptake of DDE by the aquatic midge, *Chironomus tentans* (diptera: Chironomidae). Arch. Environ. Contam. Tox. 2:152-164.

Fairchild, W. L., D. C. G. Muir, R. S. Currie, and A. L. Yarechewski. 1992. Emerging insects as a biotic pathway for movement of 2,3,7,8-tetrachlorodibenzofuran from lake sediments. Environ. Toxicol. and Chemistry (In review).

Gerould, S., P. Landrum, and J. P. Giesy. 1983. Anthracene bioconcentration and biotransformation in chironomids: Effects of temperature and concentration. Environ. Pollut. (Ser. A) 30:175-188.

Huffaker, C. B. and R. L. Rabb. 1984. *Ecological Entomology.* John Wiley and Sons, New York. pp. 315-316.

Jonasson, P. M. 1972. Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. Oikos, Suppl. 14:1-148.

Landrum, P. F., M. D. Reinhold, S. R. Nihart, and B. J. Eadie. 1985. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter. Environ. Toxicol. and Chemistry 4:459-467.

Larsson, P. 1984. Transport of PCBs from aquatic to terrestrial environments by emerging chironomids. Environ. Pollut. (Ser. A) 34:283-289.

Menzie, C. A. 1980. Potential significance of insects in the removal of contaminants from aquatic systems. Water, Air, and Soil Pollut. 13:473-479.

Nalepa, T. F., M. A. Quigley, K. F. Childs, J. M. Gauvin, T. S. Heatlie, M. P. Parker, and L. Vanover. 1985. Macrobenthos of Southern Lake Michigan, 1980-81. National Oceanic and Atmospheric Administration, NOAA Data Report ERL GLERL-28.

Timmermans, K. R. and P. A. Walker. 1989. The fate of trace metals during metamorphosis of chironomids (Diptera, Chironomidae). Environ. Pollut. 62:73-85.

(Received in USA 27 February 1992; accepted 7 April 1992)